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The Ecology of Phytoplankton with focus on Cyanobacteria and of Aquatic Macrophytes in Selected Lakes and Reservoirs of Myanmar

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Ecology of Phytoplankton with special focus on Cyanobacteria and Aquatic Macrophyte in selected lakes in Myanmar

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Abstract

Similar to other countries around the world, Myanmar's freshwater systems are increasingly subject to water quality degradation and eutrophication, and anthropogenic impacts. Dense blooms of noxious, foul-smelling phytoplankton dominated by cyanobacteria, increasing organic loading and sedimentation, fish mortalities, reduction of water clarity and degraded water quality and quantity are the most common symptoms of eutrophication in Lakes and reservoirs. Many cyanobacterial species are known to produce toxic compounds, cyanotoxins, which can affect terrestrial and aquatic organisms. However, no detailed study of the presence of cyanobacteria and their potential toxin production in the water bodies of Myanmar has been conducted so far.

This study confirms the occurrence of cylindrospermopsin (CYN) and deoxy-cylindrospermopsin (deoxy-CYN) producing *Raphidiopsis raciborskii* and microcystin (MC)-producing *Microcystis* in the cyanobacterial communities of the investigated lakes and reservoirs in Myanmar. LC-MS (LC-HRMS/MS) analysis on the MC producing strains revealed 22 to 56 MC congeners with highly toxic variants like MC-LR or MC-LA to less toxic variants like MC-RR and many new previously unidentified or unreported variants. The control of cyanobacteria is therefore vital for the management of freshwater resources.

The investigated lakes in Myanmar vary considerably in the occurrence and abundance of aquatic macrophytes. This study suggests that submerged macrophytes play an important role for the water quality and clear water conditions in a tropical shallow lake. Maintaining a high macrophyte population should therefore be a goal of lake management strategies. Based on the findings in this the study, it is highly recommended to include macrophytes and phytoplankton in the lake monitoring in Myanmar.

Key words : Cyanobacteria, cyanotoxins, aquatic macrophytes, submerged macrophyte, lake ecology, biotic interactions

မြန်မာနိုင်ငံရှိ ကုန်းတွင်းရေကန်များအတွင်း ပေါက် ရောက်လျက်ရှိသည့် ရေညှိနှင့် ရေမှော်များ၏ ဂေဟစနစ်ဆိုင်ရာ ဆက်စပ်မှုကို လေ့လာခြင်း

ဒေါက်တာသီတာဆွေ
လက်ထောက်သုတေသနအရာရှိ
သစ်တောသုတေသနဌာန၊ ရေဆင်း

စာတမ်းအကျဉ်း

ကမ္ဘာ့ပေါ်ရှိ အခြားနိုင်ငံများနည်းတူ ရေအရည်အသွေးကျဆင်းခြင်း၊ အာဟာရ ညစ်ညမ်းမှုနှင့် လူတို့၏ ဆောင်ရွက်မှုများကြောင့် မြန်မာနိုင်ငံရှိ ရေချိုစနစ်သည်လည်း ယိုယွင်း ပျက်ဆီးလျက်ရှိနေပါသည်။ အဆိပ်အတောက်ဖြစ်စေပြီး အနံ့အသက် မကောင်းသည့် စိမ်းပြာရေညှိ (ခေါ်) Cyanobacteria မျိုးစိတ် အများစုပါဝင်သည့် ရေညှိမျိုးများ ပေါက်ဖွားနေခြင်း၊ ညစ်ညမ်း အော်ဂဲနစ်ပစ္စည်းနှင့် နုန်း အနည်အနှစ်များ တိုးပွားနေခြင်း၊ ငါးမျိုးများသေဆုံးခြင်း၊ ရေကြည်လင်မှုမရှိခြင်း၊ ရေ၏အရည်အသွေးနှင့် ရေ၏ထုထည် လျော့နည်း ကျဆင်းနေခြင်းတို့သည် ရေကန် အင်းအိုင်များအတွင်း အာဟာရ ညစ်ညမ်းမှု (Eutrophication) ပြဿနာ ဖြစ်ပေါ်နေကြောင်း ညွှန်ပြနေသည့် လက္ခဏာများ ဖြစ်ပါသည်။ စိမ်းပြာရေညှိ (ခေါ်) Cyanobacteria မျိုးစိတ် အများစုသည် cyanotoxin ဟုခေါ်သည့် ကုန်းတွင်း နှင့် ရေနေသတ္တဝါများကို ဆိုးကျိုးသက်ရောက်စေနိုင်သည့် အဆိပ်အတောက် ဒြပ်ပေါင်း များကို ထုတ်လွှတ်နိုင်ကြောင်း အိမ်နီးချင်း နိုင်ငံများတွင် တွေ့ရှိထားသော်လည်း Cyano-bacteria မျိုးစိတ်များ ပေါက်ဖွားနေမှုနှင့် အဆိုပါမျိုးစိတ်များမှ အဆိပ်အတောက် ထုတ်လွှတ်နိုင်မှု အလားအလာကို လေ့လာထားသည့် သုတေသနမျိုး မြန်မာနိုင်ငံတွင် ဆောင်ရွက်ထားရှိခြင်း မရှိခဲ့ပါ။

ယခုသုတေသနစာတမ်းမှ မြန်မာနိုင်ငံရှိ သဘာဝရေကန်နှင့် ရေလှောင်ကန်များ အတွင်း Cyanobacteria မျိုးစိတ်များအနက် cylindrospermopsin (CYN) နှင့် deoxy-cylindrospermopsin (deoxy-CYN) အဆိပ် ထုတ်လွှတ်ပေးနိုင်သော *Raphidiopsis raciborskii* မျိုးစိတ်နှင့် microcystin (MC) အဆိပ်ထုတ်လွှတ်ပေးနိုင်သော *Microcystis* မျိုးစိတ်များ ပေါက်ဖွားလျက် ရှိနေကြောင်း အတည်ပြု ပေးနိုင်ခဲ့ပါသည်။ အဆိုပါ အဆိပ် ဒြပ်ပေါင်းများသည် လူနှင့် တိရစ္ဆာန်တို့၏ ကျောက်ကပ်နှင့် အသဲကို ထိခိုက် ပျက်စီး စေနိုင်

ပါသည်။ LC-MS (LC-HRMS/MS) ၏ခေတ်ခွဲ စမ်းသပ်ချက်ရလဒ်အရ MC ထုတ်လွှတ်ပေးနိုင်သည့် *Microcystis* ရေညှိမျိုးစိတ်များတွင် အလွန်အဆိပ်အတောက် ဖြစ်စေသည့် MC-LR သို့မဟုတ် MC-LA ကဲ့သို့ မျိုးကွဲများမှ အဆိပ်ဖြစ်မှုနည်းသည့် MC-RR ကဲ့သို့သော မျိုးကွဲများအပြင် အဆိပ်အတောက်ဖြစ်မှု ပမာဏကို ယခင်က လေ့လာထားရှိခြင်းမရှိသည့် မျိုးကွဲ ၂၂ ခု မှ ၅၆ ခုထိ ပါရှိကြောင်း တွေ့ရှိခဲ့ပါသည်။ ထို့ကြောင့် cyanobacteria ပေါက်ဖွားမှုကို ထိန်းချုပ်ခြင်းသည် မြန်မာနိုင်ငံ၏ ရေချိုအရင်းအမြစ်များကို စီမံအုပ်ချုပ်ခြင်းအတွက် အလွန် အရေးကြီး ပါသည်။

မြန်မာနိုင်ငံရှိ ကန်များတွင် ရေမှော်ပင်များ (aquatic macrophytes) ၏ ပေါက်ရောက်မှုနှင့် ပါဝင်မှုမှာ ကွာခြားကြကြောင်း တွေ့ရှိရပါသည်။ ဤသုတေသနမှ အပူပိုင်းရေတိမ်ကန်တစ်ခုတွင် ရေ အရည်အသွေးနှင့် ရေကြည်လင်နေမှုအတွက် ရေမြှုပ်ပင် (Submerged plants) များမှာ အဓိက အရေးပါသည့် အခန်းကဏ္ဍမှ ပါဝင်နေကြကြောင်း လေ့လာ တွေ့ရှိခဲ့ပါသည်။ ထို့ကြောင့် ကန်အတွင်း ရေမှော်ပင်များ ပေါက်ရောက်မှု ကို ထိန်းသိမ်း ထားရှိခြင်းသည် ကန်၏ ဂေဟစနစ် ရေရှည်တည်တံ့စေရေးအတွက် စီမံအုပ်ချုပ်မှု နည်းဗျူဟာ၏ အဓိက ပန်းတိုင်တစ်ခု ဖြစ်သင့်ပါသည်။ သုတေသနတွေ့ရှိချက်ကို အခြေခံ၍ မြန်မာနိုင်ငံရှိ ကန်များ၏ ဂေဟအခြေအနေအား စောင့်ကြည့်လေ့လာခြင်း လုပ်ငန်းများတွင် ရေညှိ၊ ရေမှော်များ၏ ပေါက်ရောက် နေမှုကိုပါ ထည့်သွင်း စဉ်းစားသင့်ပါကြောင်း အကြံပြုအပ်ပါသည်။

Ecology of Phytoplankton with special focus on Cyanobacteria and Aquatic Macrophyte in selected lakes in Myanmar

1. Introduction

Access to clean freshwater is important for human health, a healthy environment and poverty reduction, and it is also critical for a sustainable economy. However, similar to trends in other countries, Myanmar's water systems are increasingly subject to degradation, eutrophication, and anthropogenic impacts (Ansari et al., 2010). Common sources for water pollution in Myanmar include use of laundry detergents and soaps in lakes and rivers, poor sanitation and wastewater treatment systems, intrusion of chemical fertilizers and pesticides from agriculture, industrial waste, sedimentation, and erosion due to deforestation/forest degradation, and unsustainable agricultural practices in watershed areas (Swe, 2019). The most visible effects of eutrophication in lakes and rivers are dense blooms of noxious, foul-smelling phytoplankton, increased organic loading and sedimentation, fish mortalities, reduction of water clarity, and degraded water quality and quantity (Klapper, 1991).

Facing increased degradation of Myanmar`s freshwater systems, the National Water Resources Committee in Myanmar decided in 2014 to develop a National Water Framework Directive (NWFD), similar to the EU Water Framework Directive (EU WFD) (EC, 2000). Both the EU WFD and the NWFD have an ecosystem-based approach for characterizing and classifying water quality, an approach designed to protect, preserve and improve the aquatic environment. To assess the ecological status of surface waters, the directive requires data and indices for biological elements, supported by physical and chemical elements. Indices are important tools to assess the biological quality in lake and river habitats and are applied worldwide (Lyche-Solheim et al., 2013, Friberg et al., 2011). Indices based on aquatic macrophytes are widely applied, especially in Europe (Poikane et al., 2018, Hellsten et al., 2014, Mjelde et al., 2013, Penning et al., 2008a). Both in the NWFD and EU WFD, phytoplankton is also recognized as one of the most important bioindicators for assessing the ecological status of lakes (e.g. Nixdorf et al. 2008, Phillips et al. 2013, Nesheim et al., 2016).

Knowledge about aquatic macrophyte growth, and understanding the mechanisms behind community shifts, is of considerable importance for management aimed at promoting good ecological status of lakes and reservoirs. Aquatic macrophyte overgrowth and their management have long been a concern in Asia and other continents (Haller, 1990). The dynamics between physical and chemical water qualities and biological communities has been well studied in temperate lakes (Phillips et al., 2016, Lyche-Solheim et al., 2013), however, there is limited information for tropical lakes. It is unclear if the knowledge and conclusions from temperate systems apply to tropical lakes (Lewis, 2000).

2. Objective

Development of indices for phytoplankton and aquatic macrophytes in Myanmar requires taxonomic identification knowledge and knowledge of biological community and species response to environmental stress. However, the diversity and distribution of freshwater species in Myanmar is poorly investigated and documented (Allen et al. 2012). There is also limited or no information on the influence of macrophytes on the function of freshwater systems. More broadly, the interaction between macrophytes and phytoplankton is poorly understood in Myanmar's freshwater lakes. Various cyanobacterial species forming blooms are potential producers of hepatotoxic or neurotoxic compounds, which can be a threat to human and animal health. Toxic compounds are therefore included in the quality assessments. The use of these biological parameters should be considered in management strategies for lakes in Myanmar.

The overall goal of this study is, therefore, to provide important knowledge about the composition and abundance of phytoplankton and macrophytes and their interaction in Myanmar's freshwater systems. We focus specifically on cyanobacteria, production and presence of cyanotoxins, and aquatic macrophytes (i.e. hydrophytes, species belonging to the submerged, floating-leaved and free-floating groups as they depend largely on water quality) (Mjelde et al., 2018).

3. Literature Review

3.1 Phytoplankton

Phytoplankton are microscopic organisms comprising prokaryotic cyanobacteria and eukaryotic algae. They are free-floating organisms moving mainly with water currents. The predominant groups of phytoplankton are Bacillariophyceae, Chrysophyceae, Chlorophyceae, Cyanobacteria and Dinophyceae. Because they are sensitive to changes in environmental conditions, phytoplankton biomass and community composition can be used as indicators of water quality (Brettum and Andersen, 2005, Reynolds et al., 2002, Reynolds, 1997). The greatest biodiversity of phytoplankton organisms is often observed under low nutrient concentrations (Barcelos and Ramos et al., 2017). When nutrient concentrations are elevated, phytoplankton diversity decreases and, consequently, the phytoplankton community is mainly dominated by a few phytoplankton taxa (e.g. cyanobacteria) that are adapted to eutrophic conditions (González and Roldán, 2019, Jia et al., 2017).

3.2 Cyanobacteria

Cyanobacteria, also known as blue-green algae, blue-green bacteria, cyanoprokaryota, or cyanophytes, are mainly responsible for harmful algae blooms in lakes and reservoirs worldwide. They represent a single phylogenetic group within the domain Bacteria, with each species in this group capable of photosynthesis (Castenholz et al., 2001). They can be found in almost every terrestrial and aquatic system.

Cyanobacteria cells are spheroidal, rod-shaped, or tube-shaped. They can grow as single cells, in colonies or as straight or spiral filaments. Various cyanobacterial species are able to produce toxic compounds, called cyanotoxins. If they occur in mass developments (blooms), they can become harmful to aquatic ecosystems, the economy, drinking water supplies, recreational activities, and they can directly harm animals and humans (Paerl and Otten, 2013). Bloom-forming cyanobacteria mainly belong to the genera *Anabaenopsis*, *Aphanizomenon*, *Arthrospira*, *Dolichospermum* (*Anabaena*), *Microcystis*, *Nodularia*, *Oscillatoria*, *Planktothrix* and *Raphidiopsis* (*Cylindrospermopsis*) (Oliver and Ganf, 2000, Reynolds and Walsby, 1975). It has been estimated from water blooms and mass developments of planktic and benthic cyanobacteria in different countries that 25% to 90% of the blooms are toxic (Bláha et al., 2009, Baker and Humpage, 1994, Carmichael, 1988).

About 40 out of 150 described genera of cyanobacteria are known to produce cyanotoxins, which can affect terrestrial and aquatic organisms (Bernard et al., 2017, Van Apeldoorn et al., 2007).

3.3 Cyanotoxins

Cyanotoxins are bioactive secondary metabolites produced by cyanobacteria. Humans may be exposed to cyanotoxins via contact through recreational activities such as bathing in contaminated water and by consuming untreated, or unsuitably treated drinking water, food, or some dietary supplements (Roy-Lachapelle et al., 2017, Testai et al., 2016, Funari and Testai, 2008). These toxins can cause gastroenteritis, skin reactions and liver failure in humans (Kubickova et al., 2019, Zanchett and Oliveira-Filho, 2013, Drobac et al., 2013, Funari and Testai, 2008, Kuiper-Goodman et al., 1999), and cause skin lesions, nervous system problems, and liver damage in domestic and wild animals. Lethal effects of cyanotoxins have been reported in humans and animal such as sheep, cattle, horses, pigs and dogs (Huisman and Hulot, 2005, Cox et al., 2003, Carmichael, et al., 2001, Kuiper-Goodman et al., 1999, Jochimsen et al., 1998).

Cyanobacterial toxins are classified by how they affect the human body or their toxicological properties – e.g. hepatotoxins, neurotoxins, dermatotoxins (Codd et al., 2005, Sivonen and Jones, 1999) and according to their chemical structure such as cyclic peptides, alkaloids and lipopolysaccharides (LPS) (Chorus and Welker, 2021, Chorus and Bartram, 2005, Codd et al., 1999, Chorus and Bartram, 1999).

3.3.1 Microcystins (MCs)

The microcystins (MCs) are cyclic oligopeptides and are the most frequently found hepatotoxins in fresh and brackish waters worldwide. These toxins were named microcystins since they were first isolated from the cyanobacterium *Microcystis aeruginosa* (Kützing) Kützing (WHO, 1999, Carmichael et al., 1988). An estimated 66 % of *Microcystis* blooms produce MCs (Green, 2011).

MCs mainly affect the liver but can also affect the kidneys and reproductive system (Liu et al., 2018). MCs are inhibitors of eukaryotic protein phosphatases, which are important for cell growth and tumor suppression. Therefore, MCs are carcinogenic promoters (Carmichael, 1997, Carmichael, 1994, Luukkainen et al., 1993).

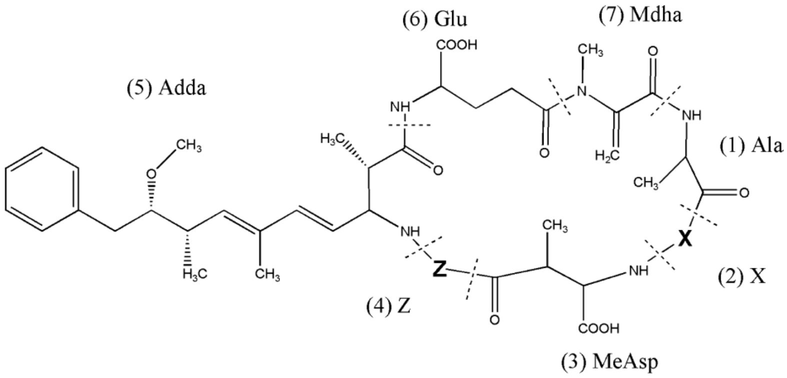

Figure 1. Chemical structure of microcystin (Kaloudis et al., 2013), cyclo (D-Ala^{1}-X^{2}-D-MeAsp^{3}-Y^{4}-Adda^{5}-D-Glu^{6}-Mdha^{7}). X and Y represent variable L-amino acids at positions 2 and 4. MeAsp is D-erythro-ß methylaspartic acid and Mdha is N-methyldehydroalanine. Adda is (2S, 3S, 8S, 9S)-3-amin-9-methox-2, 6, 8-triethyl-10-phenyl- 4, 6-decadienoic acid.

3.3.2 Cylindrospermopsin (CYN)

The cyclic alkaloid cylindrospermopsin (CYN) was first isolated from a culture of *Cylindrospermopsis raciborskii* (now *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno strains from a reservoir in tropical northern Australia (Kuiper-Goodman et al., 1999) and chemically characterized by Ohtani et al. (1992). Toxicity of CYN was first reported in 1979 on Palm Island, Australia where more than 100 persons were hospitalized.

CYN is a protein synthesis inhibitor, genotoxin (Humpage et al., 2000) and carcinogen (Falconer and Humpage, 2006) which primarily affects the kidneys, lungs, heart, thymus, spleen and intestine in mammals (Falconer et al., 1999, Hawkins et al., 1997).

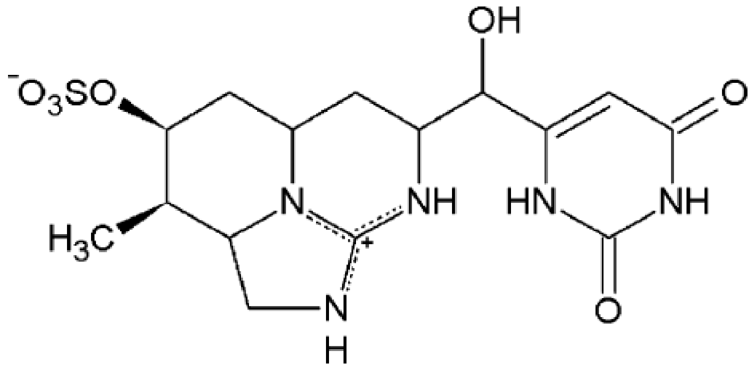

Figure 2 Chemical structure of the cylindrospermopsins (Žegura et al., 2011)

3.3.3 Anatoxin-a (ATX-a)

The neurotoxin anatoxin-a (ATX-a) is one of the most frequently found cyanotoxins and was called the very fast death factor (VFDF) due to its capacity of rapid death for animals (Bruno et al., 2017) with an LD_{50} for mice of 250 µg kg^{-1} body weight (Devlin et al., 1977). It binds to neuronal nicotinic acetylcholine receptors affecting the central nervous system. ATX-a is a bicyclic secondary amine, 2-acetyl-9-azabicyclo- [4.2.1] non-2-ene (Figure 3) (Dow and Swoboda, 2000, Carmichael, 1988, Devlin et al., 1977, Huber, 1972). Homoanatoxin-a (HANTX) is a toxic analogue of ATX-a and has an additional methyl group (CH) on carbon atom 12 (C12) (Figure 3). They both can sometimes be found together in cyanobacterial or water samples (Bruno et al., 2017, Harland et al., 2014, Mejean et al., 2009, Cadel-Six et al., 2007, Namikoshi et al., 2003) and share the same toxicological properties (WHO, 2019).

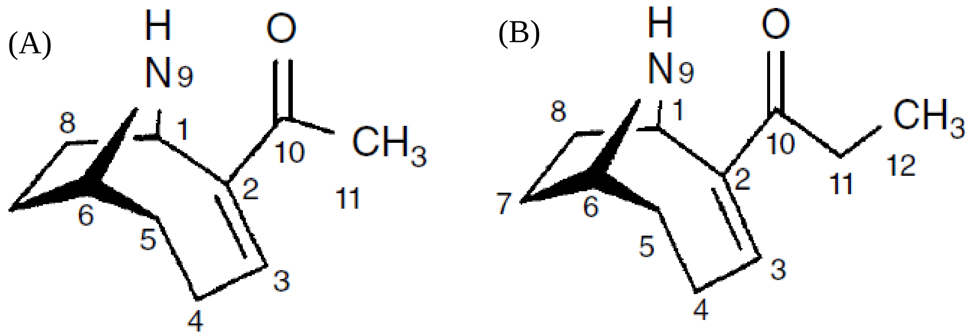

Figure 3 Chemical structures of (A) anatoxin-a (ATX-a) and (B) homoanatoxin-a (HANTX) (Bruno et al., 2017)

3.3.4 Saxitoxins (STXs)

Saxitoxins (STXs), also called Paralytic Shellfish Poisoning (PSP) toxins or Paralytic shellfish toxins (PSTs), are neurotoxic alkaloids, which are produced by marine eukaryotic dinoflagellates and freshwater prokaryotic cyanobacteria. STX has the molecular formula $C_{10}H_{17}N_7O_4$ and is composed of a 3, 4-propinoperhydropurine tricyclic system and two guanidinium groups (Figure 4), which are responsible for its high polarity (Shimizu, 2000). The alkaloids block the sodium channel in the neurons, impair the action potential, and cause paralysis of muscles (Sivonen, 1996, Kao, 1993).

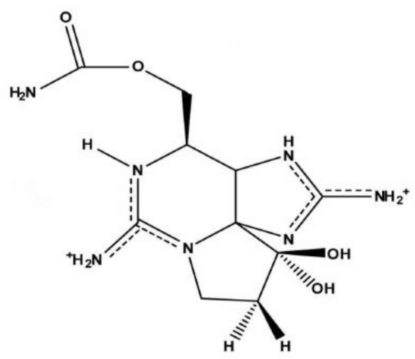

Figure 4 Chemical structure of saxitoxins (Cusick and Sayler, 2013)

Cyanotoxins have been confirmed as a causative agent in numerous reports on poisoning and mortality of animals and humans, and the prevalence of these cases is increasing through time (Codd et al., 2005, Krienitz et al., 2003, Kuiper-Goodman et al., 1999, Christoffersen, 1996, Bell and Codd, 1994). The control of Cyanobacteria therefore is vital for freshwater resource management. Aquatic phytoremediation technology is crucial for restoration of eutrophic water bodies (Yasin et al., 2017b, Straile 2015) with low cost, high ability of removing nitrogen and phosphorus and minimal disturbance to the environment (Ali et al., 2017, Wu et al, 2015). Numerous studies also demonstrated that many aquatic macrophytes could significantly suppress some cyanobacteria species (Jiang et al., 2015, Dong et al., 2014, Hilt and Gross, 2008, De Figueiredo et al., 2004). Prioritizing submerged macrophyte restoration, therefore, is an effective approach for the sustainability of lake ecosystems (Zhang et al., 2020, Hilt et al., 2006).

3.4 Aquatic macrophytes

Aquatic macrophytes are plants growing in the water submerged, floating-leaved or emergent. They can be divided into helophytes, which are semi-aquatic and emergent plants, and aquatic macrophytes (hydrophytes), which include submerged plants (elodeids, charophytes) or plants with floating leaves (nymphaeids and lemnids) (Ballot et al., 2017).

Aquatic macrophytes play a vital role in the structure and function of aquatic freshwater systems, providing refuge and food for aquatic organisms (Jeppesen et al., 2012, Timms and Moss, 1984), and improving water quality by absorbing and sequestering nutrients. Macrophytes can suppress structure and variation of phytoplankton communities and enhance water clarity through competition for nutrients (Dou et al., 2015, Mjelde and Faafeng, 1997), releasing allelopathic compounds (Gross et al., 2007), and shifting environmental light levels (Cunha et al., 2012).

High macrophyte abundance can increase a lake's resistance to increasing nutrient load (Scheffer et al. 1993a, Phillips et al., 1978). Shallow lakes without, or with only limited, macrophyte cover are more susceptible to high nutrient loads than lakes with macrophyte cover (Kuiper et al., 2017). Scheffer et al. (1993a) and Jeppesen et al. (1998) suggested two alternative stable states in temperate eutrophic lakes: a clear water state dominated by submerged macrophytes, and a turbid and phytoplankton-dominated state. The turbid state dominated by phytoplankton is considered undesirable, while water dominated by submerged macrophytes is regarded as better quality (EC, 2000).

Knowledge about aquatic macrophyte growth, and understanding the mechanisms behind community shifts, is of considerable importance for management aimed at promoting good ecological status of lakes and reservoirs. The dynamics between physical and chemical water qualities and biological communities has been well studied in temperate lakes (Phillips et al., 2016, Lyche-Solheim et al., 2013), however, there is limited information for tropical lakes. It is unclear if the knowledge and conclusions from temperate systems apply to tropical lakes (Lewis, 2000).

4. Materials and Methods

4.1 Study areas

The field survey was conducted in three water bodies: one natural lakes (Inlay Lake) and two man-made reservoirs (Meiktila Lake and Yezin Dam). The presence of toxin producing cyanobacteria and cyanotoxins were investigated in Meiktila Lake and Yezin Dam, and the assessment of the importance of aquatic macrophytes as a stabilizing factor in a tropical shallow lake was conducted in Inlay Lake.

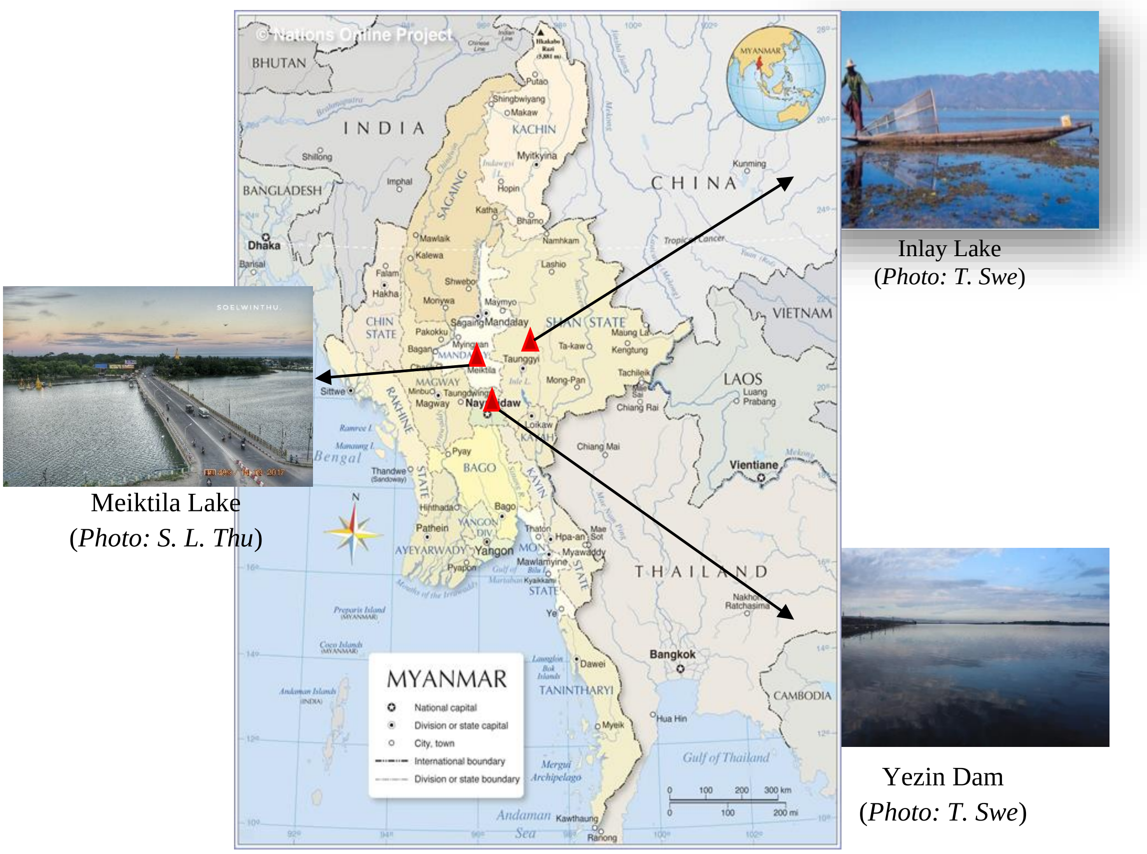

Figure 5. **Map of Myanmar with the location of the selected water bodies.** (*Source: overview-map: https://www.nationsonline.org/oneworld/map/Myanmar-administrative-map.htm*)

4.2 Methods

The methods used in the field and laboratory for this study are presented in Table (1). The cyanobacteria species and strains isolated from Meiktila Lake and yezin Dam for the cyanotoxins analysis are depicted in Table (2).

Table 1. Materials and methods used to study the physical and chemical conditions, phytoplankton, and aquatic macrophytes in freshwater lakes and reservoirs in Myanmar between 2014 and 2019.

Sr. No.	Methods
1.	In-situ measurement of water temperature, pH, Conductivity, Dissolved Oxygen
2.	Integrated samples for chemical analysis
3.	Water samples for phytoplankton
4.	Water samples for cyanotoxins
5.	Quantitative and qualitative analysis of phytoplankton using light microscopy and inverse microscopy
6.	Aquatic macrophytes survey and morphological Identification of aquatic macrophytes
7.	Isolation and cultivation of cyanobacterial strains
8.	Extraction of genomic DNA
9.	Molecular analysis of toxin producing cyanobacteria (PCR of 16S rRNA region)
10.	Molecular analysis of macrophyte (*Chara* spp.) species
11.	Construction of phylogenetic trees of cyanobacterial strains using maximum likelihood algorithm
12.	Construction of phylogenetic trees of macrophyte (*Chara* spp.) species using maximum likelihood (ML), maximum parsimony (MP) and distance (neighbour-joining (NJ))
13.	Analyses of microcystins, anatoxin-a, cylindrospermopsins, saxitoxins by ELISA
14.	Analyses of microcystins, anatoxin-a, cylindrospermopsins, saxitoxins by LC-MS/MS
15.	Statistical analysis (univariate, bivariate and multivariate analysis)

Table 2. Cyanobacteria species and strains isolated from Meiktila Lakes and Yezin Dam in Myanmar, strain codes, and ENA accession numbers.

Species	Strain	Lake/Reservoir	*Accession Number* 16S rRNA
Dolichospermum			
D. smithii	AB2017/01	Yezin Dam	LR794159
D. smithii	AB2017/02	Yezin Dam	LR794160
D. smithii	AB2017/03	Yezin Dam	LR794161
D. smithii	AB2017/04	Yezin Dam	LR794162
D. smithii	AB2017/05	Yezin Dam	LR794163
D. smithii	AB2017/06	Yezin Dam	LR794164
D. smithii	AB2017/17	Yezin Dam	LR794165
D. smithii	AB2017/18	Yezin Dam	LR794166
Raphidiopsis			
R. raciborskii	AB2017/05	Meiktila Lake	LR590626
R. raciborskii	AB2017/09	Meiktila Lake	LR590627
R. raciborskii	AB2017/12	Meiktila Lake	LR590628
R. raciborskii	AB2017/13	Meiktila Lake	LR590629
R. raciborskii	AB2017/16	Meiktila Lake	LR746263
R. raciborskii	AB2017/14	Yezin Dam	LR590626

Species	Strain	Lake/Reservoir	*Accession Number* 16S rRNA
R. raciborskii	AB2017/14	Yezin Dam	LR590627
R. raciborskii	AB2017/14	Yezin Dam	LR590628
R. raciborskii	AB2017/14	Yezin Dam	LR590629
R. raciborskii	AB2017/14	Yezin Dam	LR746263
Microcystis			
Microcystis	AB2017/14	Meiktila Lake	LR590630
Microcystis	AB2017/15	Meiktila Lake	LR590631
Microcystis	AB2017/14	Yezin Dam	LR794160
Microcystis	AB2017/15	Yezin Dam	LR794160

4. Results and Discussion

4.1 Physical and chemical properties of selected water bodies

The Inlay Lake are clear (Turbidity < 1.5 FNU) and calcareous (Ca concentrations > 40 mg/L). Water transparency was high (secchi depth to bottom) so that sunlight can reach the bottom, as in a typical shallow lake with abundant macrophytes (Scheffer and Jeppesen, 1998). Meiktila Lake is moderate alkaline (pH > 8.5; Ca = 14 – 19 mg L^{-1}) and turbid (Turbidity ≥ 83 FNU), the southern basin being more turbid than the northern. Yezin Dam is a low to moderate alkaline (pH 7.5 - 8.8) and turbid reservoir (Secchi depth = 1.5 m: Turbidity = 5.75 FNU) with large water level fluctuations. All studied lakes were characterized by average TP values of less than 20 µg L^{-1}, except Yezin Dam where higher TP values between 20 – 57 µg L^{-1} were observed during this study period. In general, the TP values of all studied lakes and reservoirs indicate oligo- to eutrophic conditions (8 – 57 µg L^{-1}) according to Salas and Martino (1991).

In contrast to earlier water chemistry studies of Inlay Lake (Pradhan et al., 2015, Akaishi et al., 2006), we found relatively low nutrient concentrations in the lake's waters. It can be assumed that a large amount of nutrients is bound in the rich macrophyte vegetation and stored in the sediments (Mjelde and Faafeng, 1997, Schneider et al., 2014, Van Donk et al., 1993). Data from the tributaries show periodically a very high nutrient input (TP= 57 – 93 µg P L^{-1}, TN = 530 – 1600 µg N L^{-1}), indicating potentially eutrophic conditions. In a recent study, higher nitrate concentrations were observed during the whole year in floating garden areas of Inlay Lake compared to the inlet streams of the lake. It was therefore concluded that lower amounts of nutrients are discharging from the watershed areas than from floating gardens (Khaung et al., 2021). We found that Inlay Lake's waters were categorized by lower electrolytic conductivity (EC) values (<300 µScm^{-1}) in the rainy season and higher EC values (> 400 µScm^{-1}) in the dry season. This is in accordance with the findings of Thin et al. (2016) and Khaung et al (2021) who observed lower EC (236 µScm^{-1} and 270 µScm^{-1}) in wet seasons and higher EC values (489 µScm^{-1} and 310 µScm^{-1}) in dry seasons.

In Meiktila Lake, we measured pH values between 8.5 and 9.3, TP concentrations between 12–23 µg L^{-1} (Phosphate = 0.4 –7.6 µg P L^{-1}) and TN concentrations between 360–570 µg L^{-1} (Nitrate = 0.83 – 8.33 µg N L^{-1}). The findings differ considerably from those in a previous study in Meiktila Lake, where lower pH values (6.5 – 7.5), higher phosphate (up to 860 µg P L^{-1}) and nitrate concentrations (up to 860 µg N L^{-1}), and wide temperature variations (23-30˚C) were recorded (Hlaing, 2014). The contradicting results between this survey and the above-mentioned studies (Khaung et al 2021, Thin et al 2016, Hlaing 2014) indicate large temporal and spatial variations of nutrient concentrations in the lakes of Myanmar. They can be explained by seasonal changes of nutrient input via inlet rivers from the upstream watershed areas and varying fertilization regimes in floating gardens in the agricultural season and off-season (Inlay Lake). Water temperature and pH of the lakes and reservoirs investigated in this study were in the same range as values observed in some tropical lakes and reservoirs in Thailand, India, Vietnam and China (Zhu et al., 2021, Quevedo-Castro et al., 2019, Bhat et al., 2015, Duong et al., 2013), while higher nutrient concentrations (N and P) were observed in the selected lakes of Myanmar.

4.2 Phytoplankton and Cyanobacteria in Selected Water Bodies

Fourteen phytoplankton groups were identified in Inlay Lake while 13 groups were recorded in Meiktila Lake and 10 groups in Yezin Dam. Bacillariophyceae, Cryptophyceae, Chlorophyceae, Euglenophyceae and Cyanobacteria were common phytoplankton groups in these water bodies.

Inlay Lake was characterized by a diverse phytoplankton composition while the phytoplankton biomass was generally very low, with average biomasses of less than 1 mg L^{-1} in all areas. The exceptions were some localities near to the shores and floating gardens where higher phytoplankton biomasses (more than 2 mg L^{-1}) of taxa like *Cryptomonas* spp., *Aphanocapsa* spp., *Merismopedia* spp. and *Monoraphidium spp.* were observed. High abundance of *Cryptomonas sp*., *Aphanocapsa sp*., *Merismopedia sp*. and *Monoraphidium sp.* is described as typical for eutrophic waters (Komárek and Anagnostidis, 1986, Huber-Pestalozzi, 1983). The floating gardens and shore areas at Inlay Lake were characterized by higher concentrations of nutrients (TP & TN) and organic matter resulting from use of fertilizers, the waste and wastewater from in-lake stilt houses, and inflowing rivers (Khaung et al., 2021, Pradhan et al. 2015, Rosén, 1981).

Relationships between macrophytes community composition and environmental variables were assessed using redundancy analysis (RDA). The analysis showed that TOC and TN significantly explain variability in phytoplankton community composition found in November 2015 in Inlay Lake. These correlations suggest that nutrients (TN or TP) are the main limiting factors of phytoplankton (including cyanobacteria) growth.

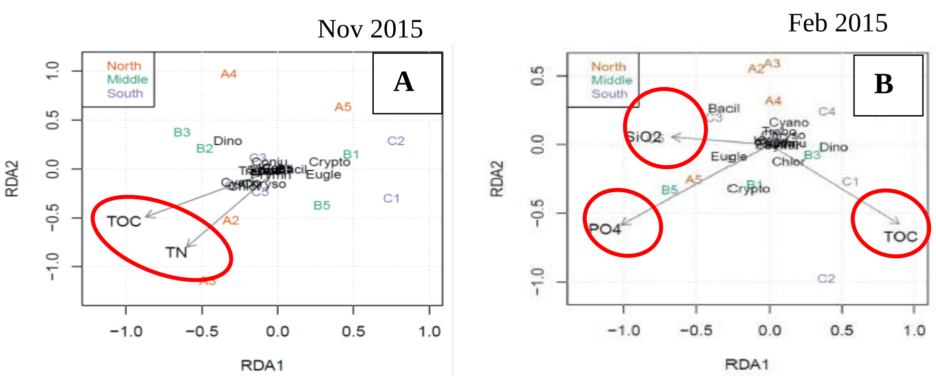

Figure 6. RDA on phytoplankton community composition from November 2015 (A) and from February 2015 (B)

In Inlay Lake, cyanobacteria were present only in smaller numbers, most likely due to the high abundance of aquatic macrophytes. In Meiktila Lake and Yezin Dam cyanobacteria were the most dominant taxa, indicating eutrophic conditions (Ke et al., 2009, Zhang and Zang, 2015). In Meiktila Lake, *R. raciborskii* was the most dominant cyanobacterial species and accounted for 27 % to 91 % (0.2 – 1.9 mg L^{-1}) of the total cyanobacterial biomass, while in Yezin Dam the amount of *R. raciborskii* was much lower with biomasses between 0.0002 and 0.2 mg L^{-1}. This species was not observed in Inlay Lake. *R. raciborskii* has not been described from Myanmar water bodies before but has been described from various other subtropical and tropical Southeast Asian freshwater habitats and other temperate water bodies worldwide (Nguyen et al. 2017, Antunes et al. 2015, Dao et al. 2010, Li et al. 2001).

Another cyanobacterial species *Dolichospermum smithii* (Komárek) Wacklin, L. Hoffmann & Komárek was the dominating taxon in Yezin Dam, with the highest biomass of 19.18 mg L^{-1}. This species was not observed in Meiktila Lake and Inlay Lake. *D. smithii* is reported of mesotrophic to slightly eutrophic ponds, reservoirs, and lakes in temperate areas but also from tropical Brazil and Senegal (Komárek, 2013, Berger et al. 2005, Sant'Anna, 1991).

Low amounts of *Microcystis* were also found in Inlay Lake and the other studied lakes and reservoirs in Myanmar. *Microcystis* has been reported from only a few water bodies in Myanmar before but is described from many Asian countries (Naw et al., 2020, Harke et al., 2016, Mowe 2015, Green 2011). Other common cyanobacteria species present in the studied water bodies were *Oscillatoria*, *Limnothrix*, *Aphanocapsa*, *Aphanothece*, and *Merismopedia*.

4.3 Cyanotoxins

During the study period, *Dolichospermum smithii* was the dominant taxon in the phytoplankton communities of Yezin Dam. However, none of the *Dolichospermum* strains isolated from Yezin Dam appeared to produce any toxins. Likewise, no strains of *D. smithii* from other locations worldwide have been shown to produce cyanobacterial toxins such as MCs, STXs, ATXs or CYNs.

ELISA confirmed that three of four *Raphidiopsis* strains from Meiktila Lake and four of five *Raphidiopsis* strains from Yezin Dam produce variable amounts of CYNs. None of the strains, however, produced STXs, ATXs or MCs. Based on ELISA analyses the concentrations of CYNs varied from 1.84 to 4.3 µg mg^{-1} fresh weight (FW) in the *Raphidiopsis* strains from Meiktila Lake and from 0.89 to 3.5 µg mg^{-1} FW in the *Raphidiopsis* strains from Yezin Dam. By using LC-MS/MS, it was shown that the CYN producing strains produced the two variants: CYN and deoxy-CYN.

The *Raphidiopsis* strains from Meiktila Lake produced CYN and deoxy-CYN concentrations between 1.7 and 2.5 µg mg^{-1} FW and 1.3 – 7.3 µg mg^{-1} FW, respectively, while the strains from Yezin Dam produced between 0.17 and 1.03 µg mg^{-1} FW and 0.11 – 2.19 µg mg^{-1} FW (Table 3).

Table 3. Cylindrospermopsin (CYN) and deoxycylindrospermopsin (deoxyCYN) concentrations in cultured *Raphidiopsis raciborskii* strains isolated from Meiktila Lake and Yezin Dam

Lake/ Reservoir	Strain	ELISA	LC-MS/MS			
		µg CYN/mg FW	**µg CYN/mg FW**	**µg deoxyCYN /mg FW**	**CYNs %#**	**deoxyCYNs %**
Meiktila	AB2017/09	2.18	-*	-*	38	62
	AB2017/05	n.d.	n.d.	n.d.	n.d.	n.d.
	AB2017/16	1.84	1.65	1.25	57	43
	AB2017/12	n.d.	n.d.	n.d.	n.d.	n.d.
	AB2017/13	4.31	2.46	7.29	25	75
Yezin	AB2017/03	0.89	0.17	0.11	61	39
	AB2017/04	3.50	0.2	0.11	65	35
	AB2017/06	-	-	n.d	n.d.	n.d.

AB2017/19	3.18	0.75	1.84	29	71
AB2017/20	1.86	1.03	2.19	32	68

*=biomass not determined, n.d. = not detected, FW = fresh weight

#=Percentages are % of total CYNs measured by LC-MS/MS.

ELISA confirmed that two *Microcystis* strains from Meiktila Lake and one *Microcystis* strain from Yezin Dam are MC producers. None of the *Microcystis* strains from Meiktila Lake and Yezin Dam produced CYNs, STXs, or ATXs. Higher MC concentration of 14000 µg g^{-1} FW was detected in the other Microcystis strain isolated from Meiktila Lake.

According to LC-MS/MS (LC-HRMS/MS) analysis on the MC producing strains, altogether 56 MC congeners including 22 previously undescribed congeners were detected in the *Microcystis* strains from Meiktila Lake and 22 MC variants including 6 previously unreported congeners were detected in the *Microcystis* strains from Yezin Dam. The toxicity of MCs produced by the isolated strains ranges from highly toxic variants like MC-LR or MC-LA to lesser toxic variants such as MC-RR (Rinehart et al., 1994). As the toxicity of several of the MC variants found in this study has not yet been investigated, a full risk assessment for these variants is difficult. Accordingly, future research should focus on the toxicity of these new variants.

4.4 Aquatic macrophytes

The investigated lakes in Myanmar varied considerably in the occurrence and abundance of aquatic macrophytes. The highest abundance of macrophytes was observed in Inlay Lake and the species richness was high when compared to other tropical lakes (Ondiba et al., 2018b, Saluja and Garg, 2017a, Dong et al., 2014, Dalu et al., 2012, Lacoul and Freedman, 2006) and even higher than in other lakes in Myanmar. Twenty-eight aquatic species comprising 16 submerged (including charophytes), 7 floating-leaved and 5 free-floating species were identified in Inlay Lake during the survey period.

The highest richness of macrophytes was recorded close to the shores, while the middle of the lake had the lowest richness. The submerged vegetation, dominated by *Nechamandra alternifolia* and *Potamogeton lucens*, and to a lesser degree, *Ceratophyllum demersum*, *Myriophyllum verticillatum*, *Najas indica* and *Chara zeylanica*, was observed throughout the year in extensive stands in most parts of the lake. The free-floating species, dominated by *Eichhornia crassipes*, formed small- to medium-sized moving islands.

The significant association of total macrophyte and submerged species abundance with spatial factors (lake areas and depth) indicate the lower abundance of submerged species in the middle and deepest part of the Lake (Figure 7), which also refer to a maximum growing depth of submerged macrophytes at approximately 3 meters in Inlay Lake.

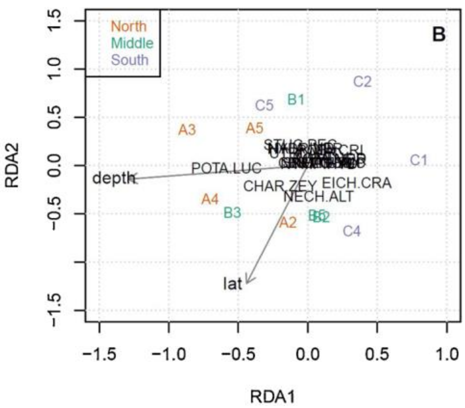

Figure 7. RDA on macrophyte community composition (February 2015).

4.5 Correlation between phytoplankton and aquatic macrophytes

Inlay Lake has been facing natural and manmade threats which have led to decreased water holding capacity, reduced water quality, hindered navigability, and reduced value to livestock (Pradhan et al., 2015, Than, 2007, Su and Jassby, 2000). The phytoplankton community composition of the lake indicates eutrophic conditions, however, it has been observed very low phytoplankton biomasses (< 1 mg FW L^{-1}) which are most likely indicating a competition for nutrients with aquatic macrophytes. It is hypothesized that submerged macrophytes control phytoplankton biomass and composition and hence stabilize a clear water state in Inlay Lake, which is supported by Yuasa et al. (2019). However, climatic conditions can vary considerably in tropical areas between rainy and dry periods, and can affect physical, chemical, and biological properties and the stability of a lake (Sahoo et al., 2016). Inlay Lake is a large, shallow lake and the composition of the macrophyte and phytoplankton communities varies considerably, both from north to south and from the littoral zone towards the center.

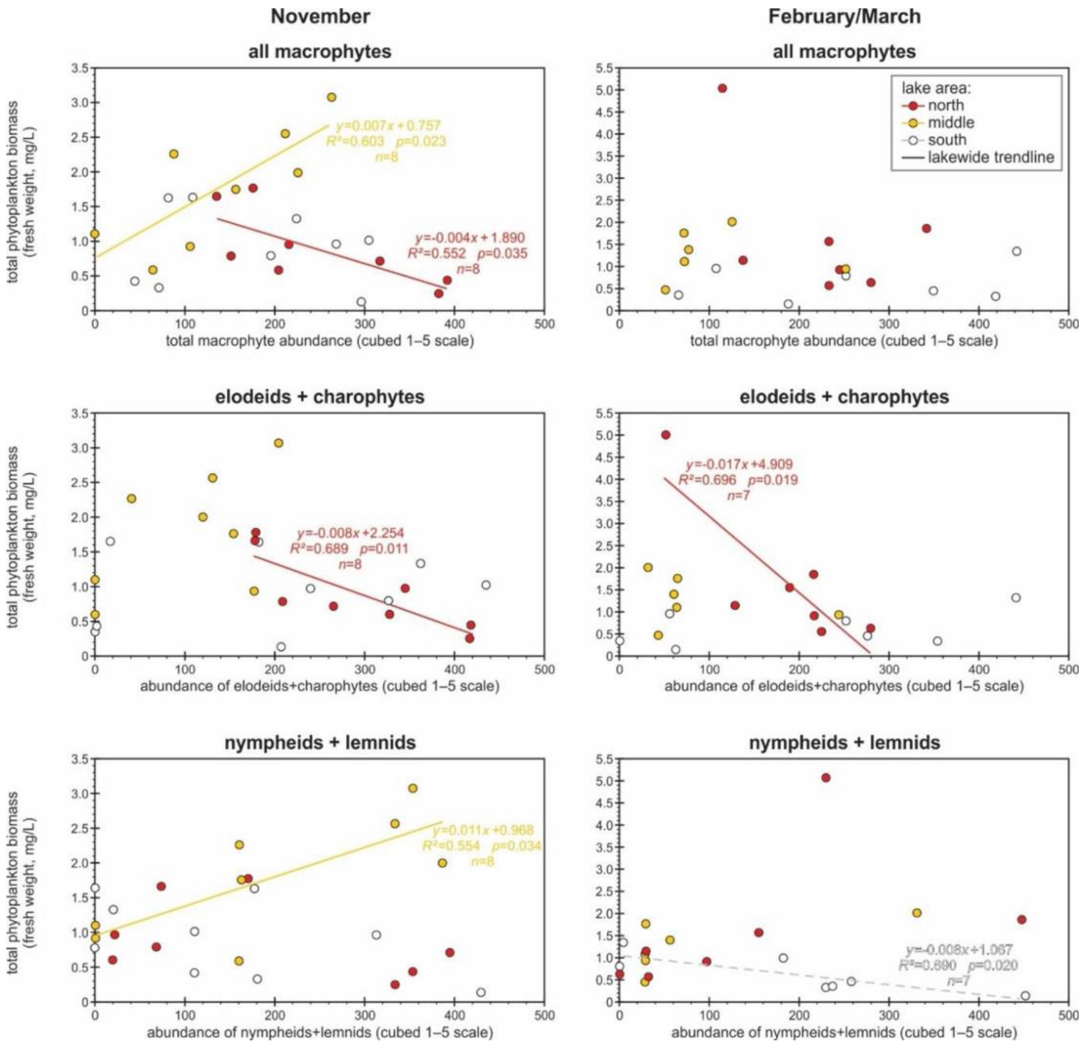

Figure 8. Relationships (as linear regressions) between total, submerged (elodeids+ charophytes), and floating-leaved (nympheids + lemnids) macrophyte abundance (cubed five-level scale) and total phytoplankton biomass by lake area and sampling period.

Total phytoplankton biomass was negatively correlated with total macrophyte abundance in the northern lake area (R = - 0.77, p = 0.03), but positively correlated (R = + 0.77, p = 0.02) in the central area in November. There were no significant correlations (p >

0.05) across sampling points in February. A negative effect of submerged macrophytes (elodeids and charophytes) on the total phytoplankton biomass was also observed in the heavily vegetated northern part of the lake at both sampling dates (R= - 0.83, p = 0.01 in November; R = 0.84, p = 0.02 in February/March). The dominant submerged macrophyte species may be capable of removing and storing large quantities of nutrients from the water by foliar and root uptake, as has been observed for temperate lakes. Several studies demonstrate how macrophytes affect the nutrient concentrations in the water and suppress phytoplankton biomasses (Hilt and Lombardo, 2010; Scheffer et al., 1993).

Floating-leaved and free-floating macrophytes (nympheids and lemnids) had a positive relationship (R = + 0.77, p = 0.03) with total phytoplankton biomass in the central lake area in November and in southern part in February/March (R = + 0.84, p = 0.02). The negative correlations (R = - 0.86, p = 0.014; R = - 0.88, p = 0.009) between cyanobacteria biomass and macrophytes, both submerged and total macrophytes, were pronounced in the northern part of the lake in February, as found in subtropical and temperate lakes (Scheffer 1998, Downing et al. 2001).

In tropical and subtropical aquatic systems, growth of floating macrophytes is high and may compete with phytoplankton for light, nutrients (nitrate, phosphate and dissolved inorganic carbon) and habitat similarly or even more than the submerged plants (Meerhoff et al., 2003, Van Donk and Van de Bund, 2002). However, we did not find a significant correlation between cyanobacteria and floating-leaved macrophytes in any part of the lake in either season. Cunha et al. (2012) showed a negative influence of free-floating and emerged species on phytoplankton in a subtropical reservoir in Brazil. A possible explanation for this discrepancy may be that, because of the low biomass of submerged macrophytes in the deeper central areas, there are enough nutrients for all macrophyte and phytoplankton groups in these areas of Inlay Lake. The very low phytoplankton biomass in the southern part of the lake and the low abundance of aquatic macrophytes is most likely related to high turbidity due to eroded material transported by the Belui River, and high boat traffic.

Several studies have shown that macrophytes affect nutrient concentrations in the water and then suppress phytoplankton growth (Hilt and Lombardo, 2010, Scheffer et al., 1993a). According to Van Donk and Van de Bund (2002) and Jasser (1995), macrophytes reduce the abundance and biomass of the phytoplankton community by modifying the phytoplankton compositions through decreasing cyanobacteria density and increasing the species diversity. Similar patterns were also observed in shallow sub-tropical lakes in Australia, China and Japan (Zeng et al., 2017, Akhurst et al., 2017, Guo-feng et al., 2000). In Maojiabu Lake in China, macrophyte restoration was used to decrease phytoplankton density and biomass and shift to phytoplankton community structures without or with a smaller number of cyanobacteria species (Zeng et al., 2017).

However, we also suspect that other factors in addition to competitive nutrient uptake are jointly responsible for very low phytoplankton biomass in Inlay Lake. For example, allelopathic compounds released from *Myriophyllum verticillatum, Ceratophyllum demersum* and *Chara zeylanica* (Hilt et al., 2006, Gross et al., 2003) and algae toxic compounds from other species like *Eichhornia crassipes* (Gross, 2003, Sharma et al., 1996) may inhibit the growth of phytoplankton. The negative correlation of cyanobacteria and submerged macrophyte in the northern part of the lake might be due to the year-round dominance of *Nechamandra alternifolia* in that area. The leaves of this species possess large secretory cells (Cook and Lüönd, 1982) and secretions from these may have an allelopathic effect on phytoplankton. However, this hypothesis is not yet confirmed. In the central lake area, the relatively low macrophyte abundance in relation to water volume might prevent the macrophytes from applying control on phytoplankton growth. The relatively high abundance of cyanobacteria found in the central lake area supports the latter hypothesis, as cyanobacteria are typically more susceptible to macrophyte allelopathy than other phytoplankton groups (Mohamed, 2017, Švanys et al., 2016, Lombardo et al., 2013, Chang et al., 2012, Gross, 2003, Jasser, 1995). This could be because the allelochemicals inhibit the photosystem II in cyanobacteria due to the oxidative stress and damaging the electron transport chain during photosynthesis (Gao et al., 2017, Zhu et al., 2010, Leu et al., 2002). It has been shown that certain macrophyte species (such as *Myriophyllum spicatum*, *Ceratophyllum demersum*, *Potamogeton malaianus*, *P. crispus, Stratiotes aloides*, *Elodea nuttallii*, *Hydrilla verticillata*, *Vallisneria spiralis*, *Najas minor*) exhibit allelopathic activity against certain phytoplankton species and inhibit their growth (such as *Microcystis aeruginosa*, *Pseudokirchneriella subcapitata*, *Anabaena flos-aquae*, *Phormidium tenue*, *Limnothrix redekei*, *Stephanodiscus minutulus*, *Scenedesmus obliquus*, *Chlorella* spp.) (Dong et al., 2018, Liu et al., 2018, Zhou et al., 2017). A similar pattern was observed in Meiktila Lake. The northern part of the lake is divided by a *Potamogeton* belt. The differences in Secchi depth and *Raphidiopsis* biomass on the two sides of the *Potamogeton* belt in the northern part of the Meiktila Lake could, therefore, be attributed to allelopathic effects of the macrophytes since water quality and other environmental factors are similar in both areas. Similar differences in Secchi depth values were also recorded by Hlaing (2014) in the same locations in Meiktila Lake.

In Yezin Dam only a few aquatic macrophytes e.g. *Hydrilla verticillata* and *Chara fibrosa* were found with low abundance during this study. This can explain the considerably higher phytoplankton (cyanobacterial) biomasses in Yezin Dam compared to Inlay and Meiktila Lakes. Since the 1990s, aquatic macrophytes in Yezin Dam have been removed to improve function and increase aesthetics (personal communication with local people). Water level regulations in Yezin Dam also impact the growth of macrophytes. The removal of macrophytes can lead to changes in phytoplankton composition and biomass, favoring the occurrence of potentially toxic cyanobacteria (Wojciechowski et al., 2018, Peretyatko et al., 2007). In Bolivian Lake Laguna it has been shown that the chlorophyll concentration has increased following the artificial removal of macrophytes (Ayala et al., 2007).

Based on the study in Inlay Lake, it appears that submerged macrophytes are important for maintaining a clear water state in tropical lakes like Inlay Lake, similar to their effects in temperate lakes (e.g., Scheffer et al. 1993b, Jeppesen et al. 1998, Hilt and Lombardo 2010, Mjelde & Faafeng 1997). Owing to differing phenology of each macrophyte species, Inlay Lake maintains robust macrophyte communities, especially of submerged species, throughout the year, and this contributes to the resilience and stability of the lake ecosystem. The extensive macrophyte abundance can store substantial amounts of nutrients, which will not be available for phytoplankton or periphyton growth. The shallowness of the lake also influences the clear water state, as the submerged vegetation covers almost the whole lake bottom and reduces turbidity.

A knowledge gap of the Inlay Lake ecosystem still exists because there is no information about zooplankton and fish communities and their grazing capacity in the lake. Several studies have shown that phytoplankton is controlled by zooplankton grazing (Williams, 1999, Stephen et al, 1998, Moss et al., 1997, Meijer et al., 1994, Knisely and Geller, 1986). Phytoplankton abundances increase in the presence of higher densities of zoo-planktivorous fish due to reduced grazing of zooplankton (Williams and Moss, 2003). Pomati et al., (2020) has shown that increasing water temperature or total grazing pressure and decreasing phosphorus levels have a positive effect on large phytoplankton groups. Fish, invertebrates, zooplankton and periphyton algae certainly play a role in the stabilization of the Inlay Lake ecosystem. The fish community of Inlay Lake includes both omnivorous and piscivorous fish species (Allen et al. 2012). The omnivorous fish may place a high predation pressure on both zooplankton and submerged macrophytes (Yu et al. 2016). To confirm the influence of zooplankton and fish on phytoplankton abundance and composition, further studies are needed.

At present, Inlay Lake is a clear-water macrophyte-dominated lake. It is stabilized by the extensive growth of submerged macrophytes of which *Chara* is is one of the dominant taxa, especially in the northern part of the lake. The presence of Charophytes in the macrophyte communities may help to buffer against the impact of nutrient inputs. Despite a high degree of human impact from anthropogenic activities such as urbanization, chemical fertilizers and pesticides, and poor sanitation, Inlay Lake maintains a low nutrient status (particularly nitrogen and phosphorus). However, lack of comprehensive management plans focussing on decreasing nutrient loads threatens the current state of Inlay Lake. Changes that may increase nutrient inputs including decreased forest cover, extensive development of hotels in watershed areas, and harvesting of macrophytes for floating garden development need to be addressed.

Increasing knowledge about the lake ecosystem is highly needed to critically evaluate the consequences of anthropogenic pressures such as nutrient loading due to increased population, agricultural areas, tourism and extension of hotel areas and sedimentation loads due to forest degradation and deforestation. Additionally, continuation of active management and monitoring of the Inlay Lake is highly recommended.

5. Conclusions

This study increases the knowledge about the composition and abundance of phytoplankton and aquatic macrophytes in the freshwater systems of Myanmar that have previously not been well investigated. It also focuses on cyanobacteria and their toxins, of selected freshwater systems, and interactions between aquatic macrophytes and phytoplankton (cyanobacteria).

The study confirms the presence of toxin producing cyanobacteria in selected freshwater bodies (both natural & man-made) in Myanmar. The common toxins in freshwater systems in Myanmar are MCs and CYNs. The high biomass of phytoplankton and the dominance of cyanobacteria with two toxin-producing taxa (*R. raciborskii* and *Microcystis*) provide evidence for a degraded state of these water bodies. Lakes and reservoirs are an important source for drinking water and irrigation in Myanmar, therefore continuous monitoring of cyanobacterial blooms and their toxins should be considered as a main activity in the integrated water resources management plan of the country (environmental and health risk assessment plan of the water bodies). Additionally, potentially harmful effects of using the water without pretreatment by humans and animals should also be considered.

This study is the first detailed whole-lake study of the interactions between phytoplankton and aquatic macrophytes and their role in shaping the Lake ecosystem in Myanmar. Maintaining or promoting aquatic macrophyte communities can be an additional and sustainable way for mitigating water quality degradation. This research shows that submerged macrophytes are the most important group for maintaining a clear water state in a tropical shallow Lake (Inlay Lake) in Myanmar. We hope that this study contributes to the understanding of the importance of aquatic macrophytes as a stabilizing factor in lakes in tropical areas. With this knowledge, it is believed that the biological management using aquatic macrophyte can be an effective measure to control cyanobacterial blooms.

Based on this study, two states of freshwater systems in Myanmar were identified: 1) eutrophic water bodies (having high cyanobacteria biomass) with limited aquatic macrophyte growth and 2) clear water systems with high phytoplankton diversity and low phytoplankton biomass (especially cyanobacteria biomass) and dominated by aquatic macrophytes. However, natural and anthropogenic influences may change the second state (i.e. clear water systems with low phytoplankton biomass and dominated by aquatic macrophytes) to a more eutrophic system. The increased practice of harvesting or physical removal of aquatic macrophytes in Myanmar's freshwater bodies (like Inlay Lake) may shift a clear water state with dense macrophyte vegetation to a turbid water state dominated by phytoplankton, especially cyanobacteria.

The success of the Myanmar National Water Framework depends on knowledge about different ecosystems, with different biodiversity and stability drivers. The examination and maintenance of biodiversity and water quality in other lakes and reservoirs in Myanmar should be given more attention. Knowledge about aquatic macrophyte growth and understanding the mechanisms behind community shifts is of considerable importance for management and for the establishment of good ecological status for lakes and reservoirs. The results in this study can be used as the basis for the ecological assessment of freshwater bodies by using phytoplankton and aquatic macrophytes as biological indicators.

Based on the findings, the following recommendations are given:

- Increased effort in ecology and management of aquatic macrophytes is critically needed for providing better outcomes for freshwater (such as water for drinking, growing crops, manufacturing, energy, transport, etc.)
- More attention should be given to the examination and maintenance of biodiversity and water quality in other lakes and reservoirs in Myanmar.
- An integrated water management plan should consider possible environmental and social impacts during the early phases of the planning process (including the wetland ecosystem, lake aquatic ecosystem, and livelihoods of people who reside in the watershed areas)
- Controlling urban runoff with treatment should be prioritized.
- All untreated drains from residential areas should be diverted or treated.
- Biological management should include control of cyanobacterial blooms.
- Preventing excessive removal (especially in Inlay Lake) or planting of macrophytes (in Yezin Dam) should be considered as a measure to help control the growth of cyanobacteria and other phytoplankton.

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